

A Novel Thaumatin-Like Protein-Encoding Gene from *Lentinula edodes*, *tlg1*, is Involved in Lentinan Degradation During Post-Harvest Preservation.

Y. Sakamoto*; M. Nagai; T. Sato

*Iwate Biotechnology Research Center
22-174-4 Narita Kitakami-shi, Iwate, 024-0003, Japan
sakamoto@ibrc.or.jp*

Lentinan, which is a β -1, 3-linked-D-glucan with β -1, 6 branches isolated as anti-tumor active-substance from *Lentinula edodes*, is purified from fresh fruiting bodies and marketed for clinical use. However, it is known that lentinan content decreases during post-harvest preservation as a result of increased β -1, 3-glucanase activity. We isolated two exo-glucanase encoding genes, *exg1* and *exg2* from *L. edodes*. Transcription level of the *exg1* and *exg2* gene was higher in the stipe than in the pileus of young fruiting bodies. This suggests that the *exg1* and *exg2* are involved in stipe elongation in *L. edodes*. We also isolated one endo-glucanase encoding gene, *tlg1*, from *L. edodes*. The *tlg1* gene had 1.0 kbp cDNA length, and encoded protein was estimated as M.W. of 25 kDa and pI value of 3.48. Putative amino acid sequence of the *tlg1* displayed 43% identity to thaumatin-like (TL) proteins from *Arabidopsis thaliana*. TLG1 had 16 cysteins conserved in TL-proteins. TL-protein is pathogen related protein 5 in plant, and several TL-protein had endo-glucanase activity. Previously, it is considered that TL-protein is unique in plant, however, this research and recent genome sequence project revealed that similar sequences to TL-proteins are conserved in filamentous fungi. We measured β -1, 3-glucanase activity of *L. edodes* fruiting bodies after harvesting by somogyi-melson method using laminarin as a substrate, and endo- β -1, 3-glucanase activity by using AZCL-pachyman as a substrate. These revealed that glucanase activity increased during post-harvest preservation. Transcription level of the *exg1* gene decreased, but the *exg2* and *tlg1* genes increased during post-harvest preservation. Western blot analysis showed that EXG2 and TLG1 expression increased after harvesting. Purified EXG1 did not degrade lentinan, but EXG2 and TLG1 degraded lentinan, therefore, we concluded that the *exg2* and *tlg1* genes are involved in lentinan degradation during post-harvest preservation.